

cal test sample may readily be determined and the concentration calculated from the standard plots.

Enzymes detectable by the assay of the present invention include, but are not limited to; oxidoreductases such as alcohol dehydrogenase, glycerol dehydrogenase, glyoxylate reductase, L-lactate reductase, malate reductase, glucose 6-phosphate dehydrogenase, mannitol 1-phosphate dehydrogenase, L-lactate dehydrogenase, glucose oxidase, galactose oxidase, L-amino acid oxidase, D-amino acid oxidase, polyphenol oxidase, ascorbate oxidase, catalase, peroxidase; hydrolases such as carboxylic ester hydrolases, cholinesterase, phosphoric monoester hydrolase, alkaline phosphatase, phosphoric diester hydrolase, phospholipase C (when the lipid used to form the liposomes is not a phospholipid); glycoside hydrolases including alpha-amylase, cellulase, lysozyme, beta-galactosidase, amyloglucosidase, beta-glucuronidase; peptidyl-amino acid hydrolase, carboxypeptidase A, peptidyl-peptide hydrolase, alpha-chymotrypsin, papain, urease, inorganic pyrophosphatase; lyases such as carbon-carbon lyases, e.g., aldehyde lyases, such as aldolase; carbon-oxygen lyases, e.g., hydrolases, such as carbonic anhydrase; carbon-nitrogen lyases, e.g., ammonia lyases, such as histidase.

The assay of the present invention can be employed in the detection and concentration calculation of circulating hormones in biological samples. Antibodies to these hormones may be raised using standard immunological techniques. Binding the antibodies to the inert solid support allows for lysis of liposomes comprised of an  $H_{II}$  forming lipid and the hormone-lipid complex. A dye or enzyme can be encapsulated in the liposome as a marker compound. These hormones include thyroid hormones such as thyroxine, triiodothyronine, parathyroid hormone and calcitonin; pancreatic hormones such as insulin, proinsulin, and glucagon; pituitary hormones including prolactin, adrenocorticotrophic hormone, tyrotropin, oxytocin, and vasopressin; uterine and placental hormones such as chorionic gonadotropin, placental lactogens, chorionic thyrotropin and relaxin; steroid hormones including estradiol, estrone, estriol, testosterone, and dihydrotestosterone; growth factors such as urogastrone, nerve growth factors and the somatomedins.

Similarly, the method may be usefully applied to the intracellular messengers, the cyclic nucleotides and prostaglandins.

The present invention may likewise be applied to the screening of circulating levels of therapeutic drugs, e.g. the cardiac glycosides; digoxin, digitoxin, anticonvulsants, diphenylhydantoin, mesantoin, phenobarbital, and mephobarbital. Of particular interest are those drugs with narrow therapeutic index, i.e., a certain minimal circulating level is required for therapeutic efficacy while a moderately higher level elicits toxic or harmful reactions.

The procedure may also be adapted to screening for antibodies raised against antibiotics, or to the antibiotics themselves, such as penicillins, cephalosporins, thienamycins, clavulanic acids, monobactams, streptomycin, and tetracyclines, chlortetracycline, oxytetracycline, and tetracycline, chloramphenicol, erythromycin, caromycin, polymyxin B. The aminoglycoside antibiotics gentamycin, amikacin, tobramycin, kanamycin and neomycin employed in the management of aerobic gram negative bacillary infections can be conveniently assayed by the present invention.

Likewise, this method may be applied to the detection and estimation of drugs of abuse such as opiates—morphine, heroin, meperidine and methadone; ergot alkaloids such as lysergic acid diethylamide; marijuana; barbiturates and cocaine and its derivatives.

The method is not restricted to small molecules. Macromolecular species including DNA, and large antigens such as egg albumin, can be directly or after conjugation with suitable lipids used to form stable bilayer liposome vesicles together with an  $H_{II}$  forming lipid. Thus, the present invention can also be applied to detection of macromolecular species such as large antigens, plasma proteins, hepatitis associated antigens, histocompatibility markers, and the like.

Since the present invention is very simple in performance and does not employ unstable or hazardous reagents, the assay method is applicable in environments which are less well-equipped and less sophisticated than typical diagnostic laboratories. For example, the assay method can be applied to screening food and environmental toxins. In food screening, important antigens would be mycotoxins and natural toxicants. This area involves such major toxins as aflatoxins, ochratoxin, patulin, penicillic acid, zearelonone; and tricothecene toxins, as well as toxic metabolites such as ipomeamerone that occur naturally in foods. In addition to the natural toxicants there are a wide variety of environmental contaminants, the presence of which in foods, even in trace amounts, poses a significant threat to mankind. These may be industrial byproducts or pesticides, e.g., polychlorinated biphenyls, chlorinated dibenzodioxins, chlorinated dibenzofurans, heptachlorepoxyde, dieldrin, and DDT, 1,1'-(2,2,2-Trichloroethylidene)-bis(4-chlorobenzene).

The present invention has been described in detail, including the preferred embodiments thereof. However, it will be appreciated that those skilled in the art, upon consideration of the present disclosure, may make modifications and improvements on this invention and still be within the scope and spirit of this invention as set forth in the following claims.

What is claimed is:

1. An immunoassay method for detecting or quantifying an analyte of interest in a test fluid, said method comprising:

- (a) forming liposomes having the analyte of interest incorporated onto the surface membrane thereof and a marker compound incorporated in the interior aqueous phase thereof;
- (b) providing a solid phase inert support having attached thereto a receptor for the analyte of interest;
- (c) mixing said test fluid with said receptor-solid phase support of step (b) for sufficient time to saturate said receptor with any analyte present in said test fluid;
- (d) mixing said liposomes formed in step (a) with said saturated receptor-solid phase support from step (c) causing the lysis of said liposomes, without the addition of any membrane lytic molecules, ions, or active complements, and;
- (e) determining the presence of marker compound released by the liposomes in step (d).

2. The immunoassay of claim 1 wherein step (e) further comprises quantifying the amount of marker compound released and determining the amount of analyte present in the test fluid.

3. The immunoassay of claim 1 wherein said analyte of interest is an antigen.